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TEMPERATURE EFFECTS ON THE DEVELOPMENTAL RATE OF SQUID (*LOLIGO PEALEI*) EMBRYOS[1]

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As is usual for invertebrates, the rate of embryonic development of some cephalopods appears to be temperature dependent. Hamabe (1960) reported total developmental times of 36–43 days at 13–17° C and 46 days at 10–12° C for *Loligo bleekeri*. Developmental time of *Loligo opalescens* ranged at least from 22–24 days at 16° C (Fields, 1965) to 30–35 days at 13.6° C (McGowan, 1954). Choe (1966) reported that the developmental times of five species of squid and cuttlefish were highly dependent on water temperature. Costello, Davidson, Eggers, Fox and Henley (1957, pages 155–159) warned that their timetable of development for *Loligo pealei* might vary considerably with water temperature.

The squid, *L. pealei,* comes inshore near Woods Hole, Massachusetts in April and remains through November (Sumner, Osburn and Cole, 1913; Summers, 1968, 1969, 1971 and unpublished). Squid eggs are usually available in the Woods Hole area from May to October (Verrill, 1882; Bumpus, 1898; Drew, 1911; Summers, 1968, 1969, 1971 and unpublished) during which time sea water temperatures range from approximately 10 to 23° C, with a maximum in mid-August. Salinity range is approximately 30–32‰ during this period. The common occurrence of squid eggs and the large, natural temperature range facilitated a study of the relationship between sea water temperature and the developmental rate of *L. pealei* at Woods Hole.

Materials and Methods

Squid, *L. pealei,* eggs were collected within 20 km of Woods Hole by otter trawl on the following dates in 1970: May 11, May 14, May 20, July 14, and September 24. Some eggs, deposited in laboratory tanks by freshly caught squid, were collected on: May 27, June 4, June 8, July 30, and August 13. Dr. John M. Arnold donated the eggs of July 30 and August 13. A total of 18 sets of egg strings were obtained by otter trawl and 19 sets were obtained from laboratory tanks. The source of squid eggs was not judged important because only healthy embryos were used in our experiments.

Squid egg strings contain 150 to 200 embryos each (Williams, 1909). Individual egg strings were examined, compared to Arnold's (1965) normal embryonic stages and grouped by developmental stage. Each set (4 to 6 egg strings from the same source and matched for developmental stage) was provided flowing sea

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water in a new 20 cm stacking dish lined with an open-ended cylinder of 1.4 mm nylon mesh. The mesh extended 5 to 10 cm above the rim and retained egg strings and freshly hatched squid.

Ambient temperature sea water, from the laboratory sea water system, was supplied to stacking dishes through glass and rubber tubing. Chilled sea water, from the laboratory chilled sea water system, filled a seasoned fiberglass reservoir. Glass and rubber tubing siphons carried water from the reservoir to stacking dishes. Sea water flow was sufficient to circulate egg strings in each dish. In our experiments, nearly all eggs provided with flowing sea water hatched. Development terminated in a few cases when the sea water flow stopped and the water became stagnant. These sets were discarded and the data were not included in our results.

One randomly chosen egg string was removed from each dish and observed under a dissecting microscope on an average of once every 36 hours. At each observation at least 20 embryos were staged using Arnold's (1965) scale of stages; time, sea water temperature, and dominant developmental stage were recorded. Stage 30 (hatching) was recorded at the first occurrence of newly hatched squid. Seventeen sets of egg strings were observed in ambient temperature sea water. Twenty additional sets were sub-divided into ten matched pairs: one set of each pair in ambient sea water and one in chilled sea water. We made a total of 377 observations which represent approximately 7540 determinations of embryonic stages.

Results

Plots of developmental stage *vs.* time before hatch were prepared for each set of egg strings; these were compared and grouped by similarity of developmental time course. Three distinct groups resulted, each with a specific temperature range as shown in Figure 1. Group I included all ten sets of egg strings from chilled sea water and nine sets from ambient sea water. Embryos in chilled sea water at 13.0 to 16.9° C exhibited a time course of development indistinguishable from that of embryos in ambient sea water at 12.0 to 18.0° C. Mean developmental time for Group I embryos was 642 hr. The ten sets of egg strings in Group II were kept in ambient sea water at 15.5 to 21.3° C; mean developmental time was 445 hr. Group III included eight sets of egg strings in ambient sea water at 21.5 to 23.0° C; mean developmental time was 257 hr. In all groups survivorship to hatching approached 100%.

Embryonic development appeared to consist of four phases. The first three phases each required a specific proportion of the total developmental time independent of temperature, but the fourth phase (hatching) required a specific time interval independent of temperature. Development to stage 12 was non-linear and required 13.2% (SD 3.4%) of the total developmental time. Embryos developed from stage 12 to stage 26 at a linear rate which occupied 51.3% (SD 5.5%) of the total time. Linear development from stage 26 to stage 29 required 22.0% (SD 3.2%) of the total time. Development from stage 29 to stage 30 (hatching) apparently required 52.0 hr (SD 4.0 hr) independent of temperature.

Figure 2 shows the total developmental time (deposition to hatching) plotted against sea water temperature for all data groups. Smooth curves were fit by inspection. The following temperature indices are included: maximum temperature, mid-range temperature, weighted mean temperature, and deposition tempera-

ture. The latter corresponded closely with the minimum temperature for each group because the sea water was warming seasonally during the experiments. Also included in Figure 2 are data from the literature on *L. pealei* by Arnold (1965), Bruce (1886) and Costello *et al.* (1957).

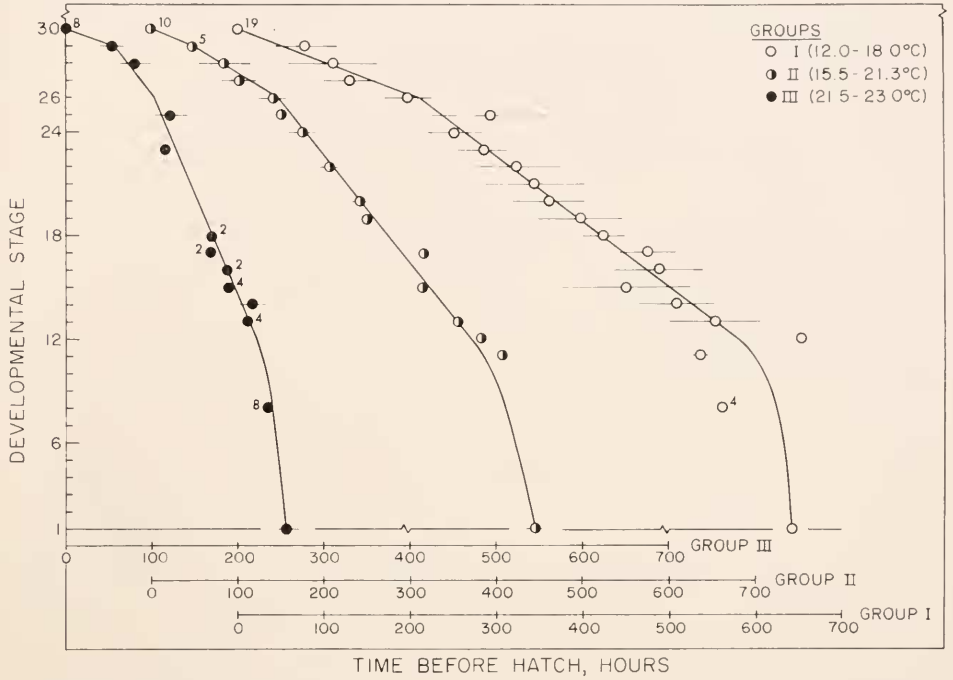

FIGURE 1. Mean and standard deviation of time before hatch at observed developmental stages (after Arnold, 1965) for each data group. The number of observations per point ranged from 1 to 33, with a mean of 7. Where more than one observation of a developmental stage occur at the same time before hatch, the number of such observations is shown beside the point. The horizontal scale has been displaced 100 hr between groups for clarity in presentation.

DISCUSSION

Our data show a direct relationship between sea water temperature and developmental rate to stage 29. The plot of deposition temperature *vs.* total developmental time (Fig. 2) is useful for predicting the approximate hatching time of squid embryos in seasonally warming sea water. If the deposition temperature is not known or if the sea water temperature is altered artificially (*e.g.*, when eggs are placed in chilled sea water), the approximate hatching time can be predicted by extrapolation from an observed time interval between established stages. At low temperatures, observed time of first hatch may differ from the predicted value by as much as 2 or 3 days due to heterochrony within individual egg strings.

The scatter of data summarized in Figure 2 does not permit extensive comparisons of developmental rate with published information on other animals, largely because of variation in ambient sea water temperatures. In our experiments, the

total developmental time was approximately a linear function of mid-range temperature, and this relationship can be extrapolated to (an impossible) "zero" developmental time at about 27° C. Extrapolation of the seasonally varying deposition temperature suggests a minimum developmental time at nearly the same temperature. We cannot predict a temperature for "zero" developmental rate due to lack of data at temperatures below 12° C. Attempts to calculate the Bělehrádek

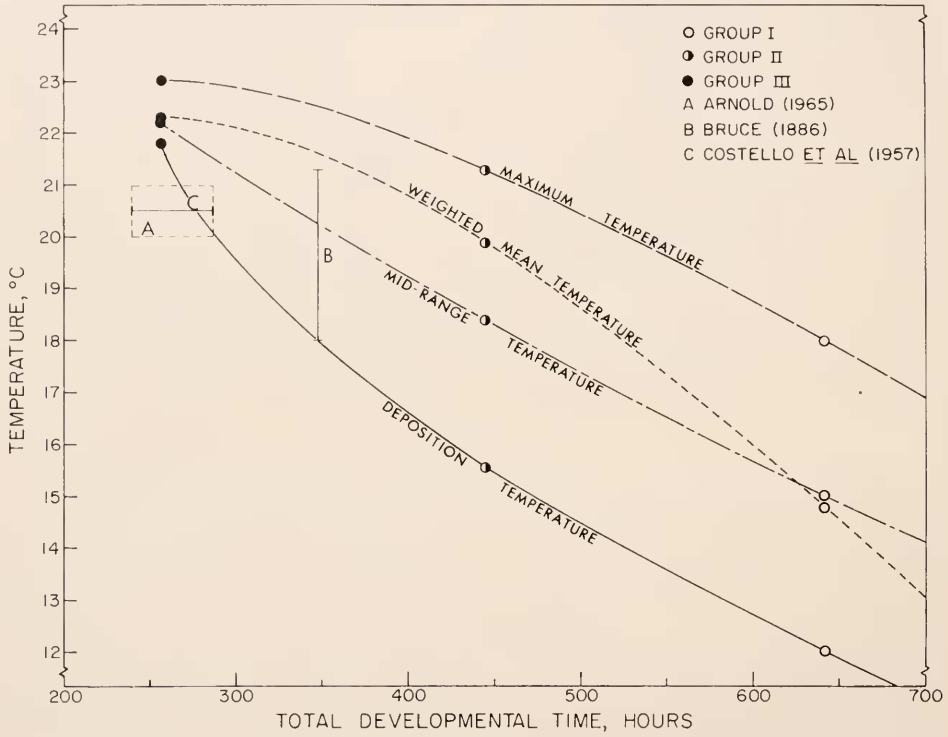

FIGURE 2. Total developmental time of *Loligo pealei* embryos as related to sea water temperature. Data from Arnold (1965), Bruce (1886) and Costello *et al.* (1957) are also included. Arnold (dashed box) reported a total developmental time of 240 to 288 hr for embryos at 20 to 21° C. Bruce (vertical line) reported that eggs deposited on July 3 hatched on July 18 (1886?), a total developmental time of approximately 348 hr. Costello *et al.* (horizontal line) reported a total developmental time of 240 to 288 hr. Bruce and Costello *et al.* provide no temperature data for their work at Woods Hole. Bruce's data are plotted between the temperatures (18.0° C, 21.3° C) observed on July 3 and July 18, 1970. Data from Costello *et al.* are plotted at the temperature (20.5° C) observed on July 15, 1970, because of the statement that most female squid have bred by mid-July.

"biological zero" temperature as used by McLaren (1966) were inconclusive. We do suggest that the natural occurrence of squid eggs would be limited to deposition temperatures at which adult squid are likely to be found, especially temperatures of at least 8° C (see Summers, 1969 and 1971). Thus, *L. pealei* eggs may fully develop over nearly a 20° span of sea water temperature, though we have verified this only over an 11° range.

The chronology of developmental stages at various sea water temperatures requires further examination. With the exception of early stages (corresponding to cellular events now classical in embryology) and hatching, Arnold's (1965) descriptions of normal embryonic stages were based on arbitrarily chosen, visible features not necessarily separated by consistent time intervals. As a result, stage rates of development should only be compared at the same stage between sets of embryos or over a number of consecutive stages for any one set of eggs. The latter is our justification for indicating linear stage rates and phases for portions of the data shown in Figure 1. As reported above, the developmental phases progressed in proportion to the total developmental time for all temperature groups with the exception of phase 4 (hatching). The inconsistency may be trivial because stage 30 was experimentally difficult and its first appearance probably represents a minimal hatching time.

Arnold's (1965) stage chronology clearly indicates a significant change in stage rate around stage 12 corresponding to the intersection of our phases 1 and 2. His data do not confirm our separation of phase 3 at stage 26. Broadly speaking, our phases relate to the following embryonic occurrences: (1) cellular events on the yolk surface, (2) differentiation, (3) growth at the expense of yolk and (4) hatching. The organic content of squid embryos has been reported by Russell-Hunter and Avolizi (1967). They demonstrated a relatively small increase in nitrogen and organic carbon, a greater relative increase in water content (wet weight minus dry weight) and a relatively large increase in salt content (inorganic ash) during the development of *L. pealei*. No significant uptake was reported during phase 1. The stage rate of salt uptake relative to water content dropped considerably between phases 2 and 3 possibly indicating the initiation of organ functioning and/or ionic regulation. This change is reflected in post-hatched values of 65% for organic components (carbon and nitrogen) and 21% for inorganic ash compared with stage 29 (Russell-Hunter, personal communication). These data suggest a functional distinction of phase 3.

Verrill (1882), Costello *et al.* (1957), Arnold (1965 and personal communication) and Russell-Hunter (personal communication) observed hatching of advanced (stage 29) *L. pealei* embryos caused by a mechanical stimulus. Fields (1965) considered low light intensity important for hatching of *L. opalescens*. Choe (1966) reported that a mechanical stimulus or a sudden change in either water temperature or salinity could elicit hatching in five cephalopod species. We observed hatching of advanced *L. pealei* embryos probably caused by an increase in water temperature or by a mechanical stimulus, but we did not specifically study any external hatching stimulus.

Squid in the vicinity of Woods Hole breed primarily from mid-May through June but some breeding continues into September (Verrill, 1882; Costello *et al.*, 1957; Summers, 1968, 1969, 1971 and unpublished). Due to sea water temperature differences, eggs deposited in May develop more slowly than eggs deposited in June. (In our experiments, eggs deposited on May 22 and June 3 hatched on June 18 and 21, respectively.) In an attempt to relate developmental temperature effects to the observed size distribution of young squid (Summers, 1967 and 1971), artificial size distributions were constructed by a Monte Carlo Method. Hatching size was assumed to be 1.8 mm dorsal mantle length which was the mean

value from 88 measurements of newly hatched squid on two separate dates and corresponds with Arnold's (1965) scaled drawing of stage 30 squid. We assumed a normal distribution of egg deposition and a linear growth rate after hatching. Dates of mean egg deposition were selected at two-week intervals from May 15 to June 30. Sea water temperature data for Woods Hole (kindly provided by Mr. Charles L. Wheeler of the National Marine Fisheries Service, Biological Laboratory, Woods Hole) was used with Figure 2 to predict hatching dates. Constructed size distributions were skewed toward smaller animals; observed size distributions of young squid were skewed toward larger animals. We concluded that the size distribution of young squid was not simply the result of developmental temperature. Other factors, including the time distribution of egg deposition, vertical distribution of newly hatched squid, predation pressure and planktonic dispersal probably affect the measured size distribution of young squid.

Fields (1965) reported the polychaete *Capitella ovincola* from the intermediate jelly of *L. opalescens* egg strings. *Capitella hermaphrodita* lives and reproduces in the jelly of *Loligo vulgaris* egg strings (von Boletsky and Dohle, 1967). We did not observe any polychaete or other macroscopic commensal in *L. pealei* egg strings.

Chromatophores become evident at stage 26, the beginning of phase 3 (Arnold, 1965). Newly hatched squid have active chromatophores of three distinct colors: red, brown and light green which become pink, reddish brown and light green, respectively, when expanded. The latter becomes orange (yellow expanded) within a few days after hatching. Two large reddish brown chromatophores are located on the dorsal mantle surface between the fins. These remain expanded for at least a week after hatching (the maximum survivorship in our experiments).

In the laboratory under all conditions of illumination, newly hatched squid expend considerable amounts of energy to remain at or near the water surface. Repeated failures to collect numbers of young squid with surface plankton nets suggest that this behavior is a laboratory artifact.

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Summary

1. *Loligo pealei* embryos were readily maintained in flowing sea water between 12.0 and 23.0° C. They were observed and staged according to Arnold's (1965) description of normal embryonic development. Developmental stage *vs.* time plots fell into three groups with sea water temperature ranges of 12.0–18.0° C, 15.5–21.3° C and 21.5–23.0° C and mean total developmental times of 642, 445 and 257 hr, respectively.

2. The rate of development appeared to be directly related to sea water temperature and could be modified at any stage by altering sea water temperature. Extrapolations of this relationship are possible, and practical limits of its extension are discussed.

3. Development apparently consisted of four phases; the first three each requiring a specific proportion of the total developmental time independent of tempera-

ture and the fourth (hatching), requiring a specific time interval independent of temperature. Development to stage 12 was non-linear and required 13% of the total time. Linear development over stages 12 to 26 required 51% of the total time. Stages 26 to 29 also developed linearly, but required 22% of the total time. In each group, development from stage 29 to stage 30 (hatching) required approximately 52 hr, apparently independent of temperature. Functonal distinctions are suggested for these developmental phases.

4. Approximate time of first hatch could be predicted from the sea water temperature at egg deposition or from an observed time interval between established stages during development.

5. Artificial size distributions constructed from the developmental data and observed sea water temperatures differed markedly from measured size distribution of young squid. Factors other than temperature probably affect the measured size distributon of young squid.

LITERATURE CITED

Arnold, J. M., 1965. Normal embryonic stages of the squid, *Loligo pealii* (Lesueur). *Biol. Bull.*, **128**: 24–32.

Boletzky, S. von, and W. Dohle, 1967. Observations sur un capitellidé (*Capitella hermaphrodita* sp. n.) et d'autres polychétes habitant la ponte de *Loligo vulgaris*. *Vie Milieu, Serie A*, **18**(1–A) : 79–98.

Bruce, A. T., 1886. Segmentation of the egg and formation of the germ layers of the squid (*Loligo pealii*). *Johns Hopkins University Circulars*, **2**(54) : 45–46.

Bumpus, H. C., 1898. The breeding of animals at Woods Holl during the month of May, 1898. *Science*, **8**(185) : 58–61.

Choe, Sang, 1966. On the eggs, rearing, habits of the fry, and growth of some Cephalopoda. *Bull. Mar. Sci.*, **16**: 330–348.

Costello, D. P., M. E. Davidson, A. Eggers, M. H. Fox and C. Henley, 1957. *Methods for Obtaining and Handling Marine Eggs and Embryos*. Marine Biological Laboratory, Woods Hole, 247 pp.

Drew, G. A., 1911. Sexual activities of the squid, *Loligo pealii* (Lesueur), I. Copulation, egg laying and fertilization. *J. Morphol.*, **22**: 327–359.

Fields, W. G., 1965. The structure, development, food relations, reproduction, and life history of the squid *Loligo opalescens* Berry. *Fish. Bull., California Fish and Game*, **131**: 1–108.

Hamabe, Mototsugu, 1960. Observations of early development of a squid, *Loligo bleekeri* Keferstein. *Ann. Rept. Jap. Sea Reg. Fish. Res. Lab.*, (**6**): 149–155.

McGowan, J. A., 1954. Observations on the sexual behavior and spawning of the squid *Loligo opalescens* at La Jolla, California. *California Fish and Game*, **40**: 47–54.

McLaren, I. A., 1966. Predicting development rate of copepod eggs. *Biol. Bull.*, **131**: 457–469

Russell-Hunter, W. D., and R. J. Avolizi, 1967. Organic content in developing squid eggs assessed from carbon, nitrogen, and ash, and its evolutionary significance. *Biol. Bull.*, **133**: 470–471.

Summers, W. C., 1968. The growth and size distribution of current year class *Loligo pealei*. *Biol. Bull.*, **135**: 366–377.

Summers, W. C., 1969. Winter population of *Loligo pealei* in the mid-Atlantic Bight. *Biol. Bull.*, **137**: 202–216.

Summers, W. C., 1971. Age and growth of *Loligo pealei*, a population study of the common Atlantic Coast squid. *Biol. Bull.*, **141**: 189–201.

Sumner, F. B., R. C. Osburn and L. J. Cole, 1913. A biological survey of the waters of Woods Hole and vicinity. *Bull. U. S. Bur. Fish.*, **31**(2) : 549–794.

Verrill, A. E., 1882. Report on the cephalopods of the northeastern coast of America. *Rep. U. S. Comm. Fish.*, (Part 7), **1879**: 211–455.

Williams, L. W., 1909. *The Anatomy of the Common Squid*, Loligo pealii, *Lesueur*. Brill, Leiden, 92 pp.